

Figure 1. Hypersensitivity of Gyrase Mutant Alleles

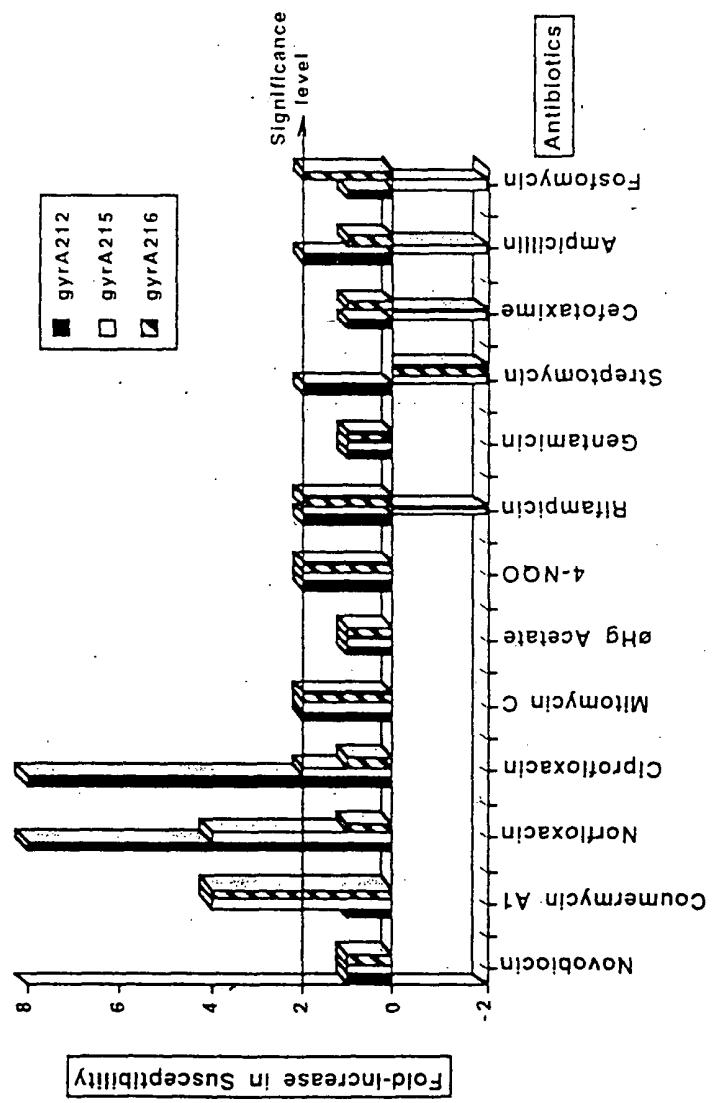


Figure 2. Hypersensitivity Profiles of *Salmonella* Ts Mutants

	Gyrase Inhibitors			DNA/RNA metabolism			Protein metabolism			Cell wall inhibitors		
	Nov	Cou	Cipro	Nor	MitoC	NQO	Rif	Gen	Strep	Phen	Cefo	Amp
5155	dnAE	-	-	-	-	-	-	-	-	-	-	4
	gyrA16	-	-	-	-	-	-	-	-	-	-	-
	gyrA215	-	-	-	-	-	-	-	-	-	-	-
	gyrA22	-	-	-	-	-	-	-	-	-	-	-
7784	parC	-	-	-	-	-	-	-	-	-	-	-
	clm?	-	-	-	-	-	-	-	-	-	-	-
	parE	-	-	-	-	-	-	-	-	-	-	-
	parE	-	-	-	-	-	-	-	-	-	-	-
	parF	-	-	-	-	-	-	-	-	-	-	-
	parF	-	-	-	-	-	-	-	-	-	-	-
	parF	-	-	-	-	-	-	-	-	-	-	-
	clm?	-	-	-	-	-	-	-	-	-	-	-
	murB	-	-	-	-	-	-	-	-	-	-	-
	Round	-	-	-	-	-	-	-	-	-	-	-
7587	dapA	-	-	-	-	-	-	-	-	-	-	-
3319	murE	323	3264	328	-	3164	3642	3683	-	3743	-	-
5081	Thy Irc	2842	3264	-	-	369	3643	3644	-	3645	-	-
7585	Odd	-	-	-	-	-	-	-	-	-	-	-
5208	fsH	-	-	-	-	-	-	-	-	-	-	-
7141	Filam	-	-	-	-	-	-	-	-	-	-	-
5052	Filam	-	-	-	-	-	-	-	-	-	-	-
5051	Filam	-	-	-	16	-	64	4	-	-	-	4
5041	UV-	-	-	-	-	16	4	4	-	-	-	-
5086	UV-	-	4	-	+4	-	32	4	-	-	-	32
	clm?	-	-	-	-	-	-	-	-	-	-	8

\* indicates that there was no significant difference with the wild type parent strain. \*ND\*: Not determined.

Abbreviations: Nov, novobiocin; Cou, coumermycin; Cip, ciprofloxacin; Nor, norfloxacin; MitoC, mitomycin C; Cltg, phenylmercuric acetate; NQO, 4-nitroquinoline oxide; Rif, rifampicin; Gcn, gentamicin; Strep, streptomycin; Phen, phenol; Cef, cefotaxime; Amp, ampicillin; Fosf6, fosfomycin; clm?, unknown conditional lethal mutant; Round, round cell morphology; Thy inc, defective thymidine incorporation phenotype; Odd, odd cell shape morphology; Filam, filamentous cell morphology; UV, UV sensitive. Known or closely related genotypes: *thrA*, DNA polymerase III  $\alpha$  subunit; *gyrA*, gyrase subunit A; *parC*, Gyra-like subunit of Topoisomerase IV; *parE*, GyrB-like subunit of Topoisomerase IV; *parF*, Acetyl transferase activity, associated with the topoisomerase IV gene; *murB*, UDP-N-acetylglucosaminyl-3-enolpyruvate reductase; *dapA*, Dihydrodipicolinate synthase; *murCEFG*, near cluster of *L*-Ala, DAP, D-Ala-D-Ala and NAG ligases; *fsH*, maybe *fsH* by map location.

**Figure 3. Potential Relationships Among Essential Genes**

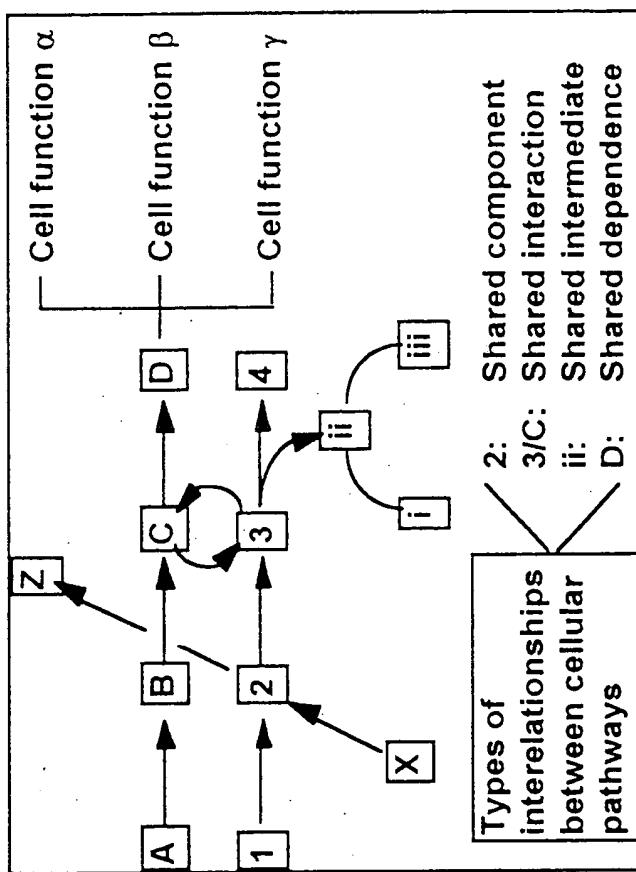


Figure 4. Single and Multi-Score Multichannel Screen

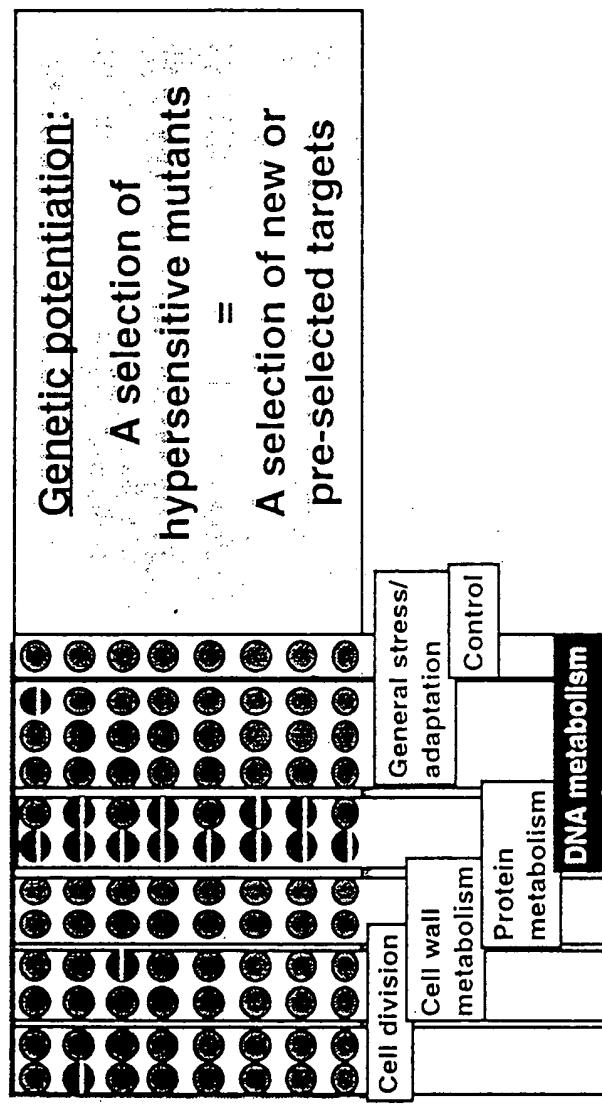


Figure 5. Operational Design of Multichannel Screen

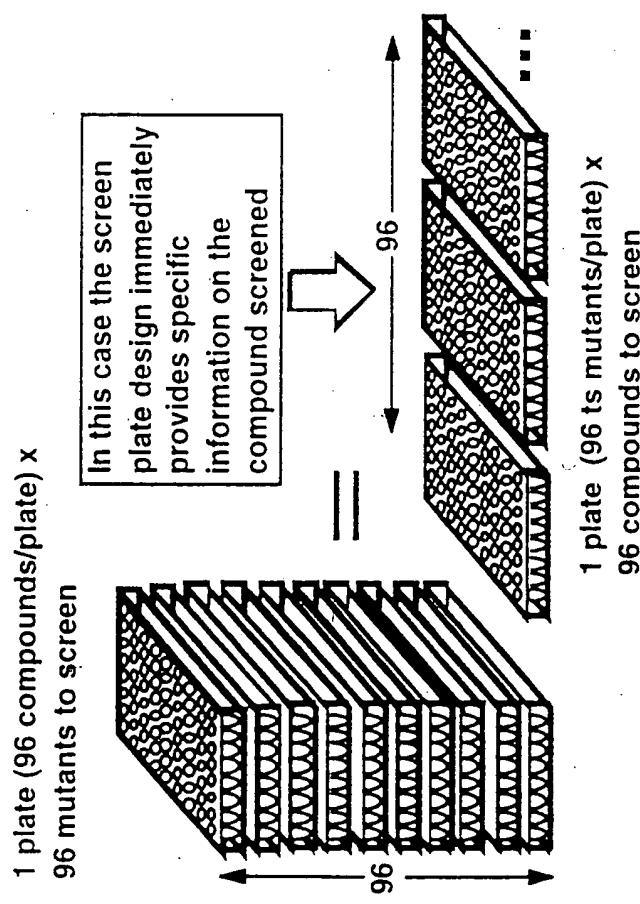


Fig 6

# Heat-Sensitivity Profiles of TS<sub>1</sub> Mutants in Different Genes

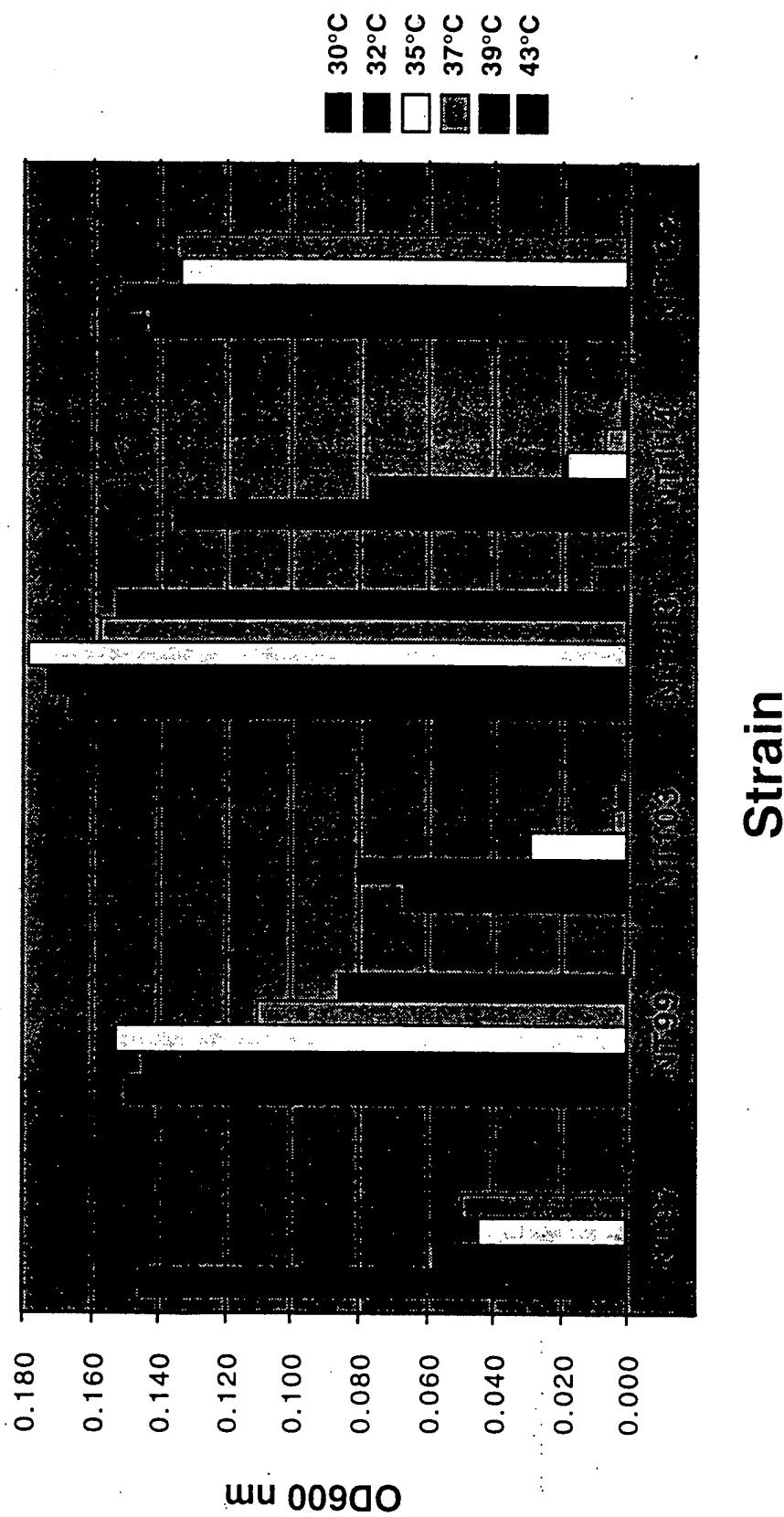
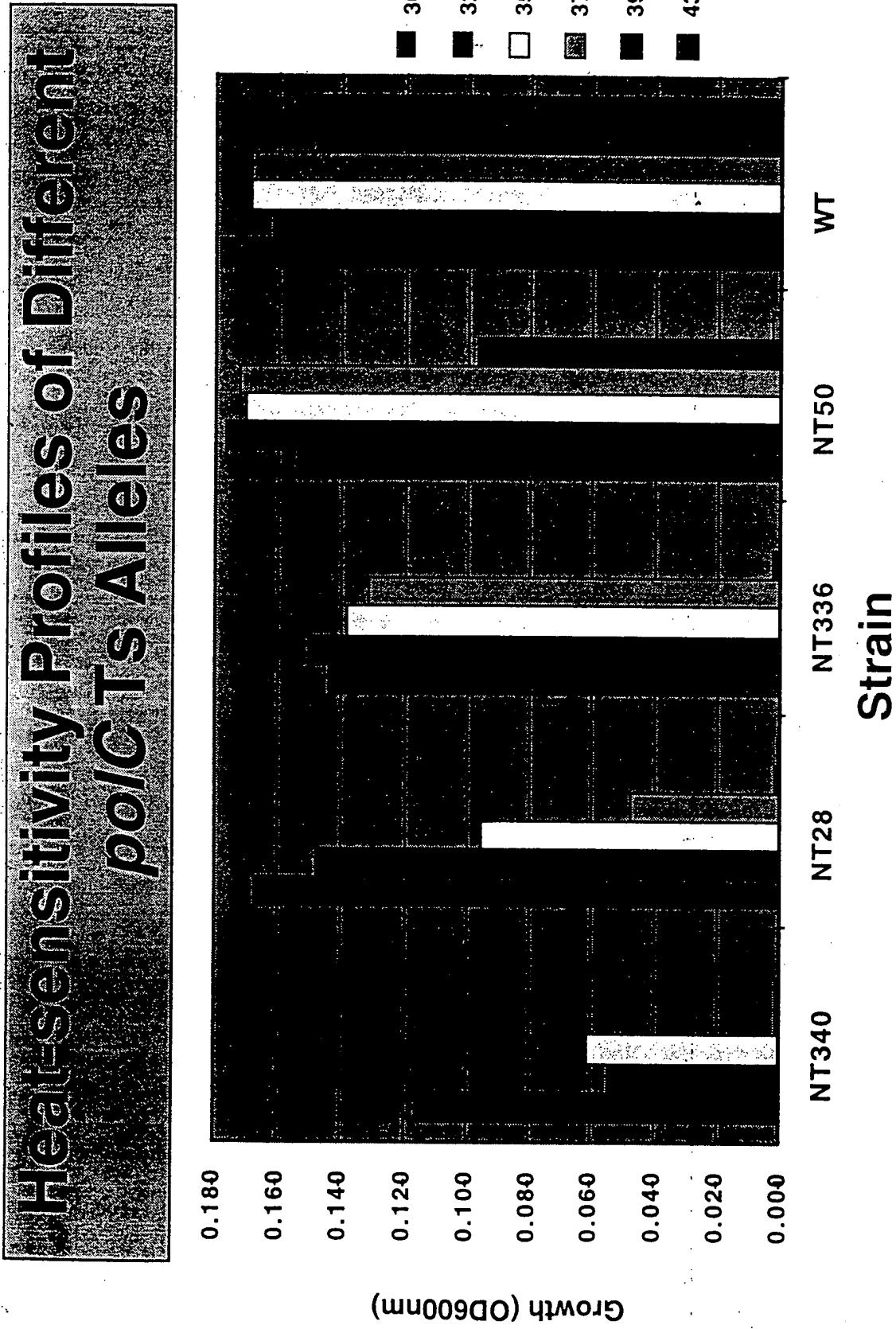


Fig 7



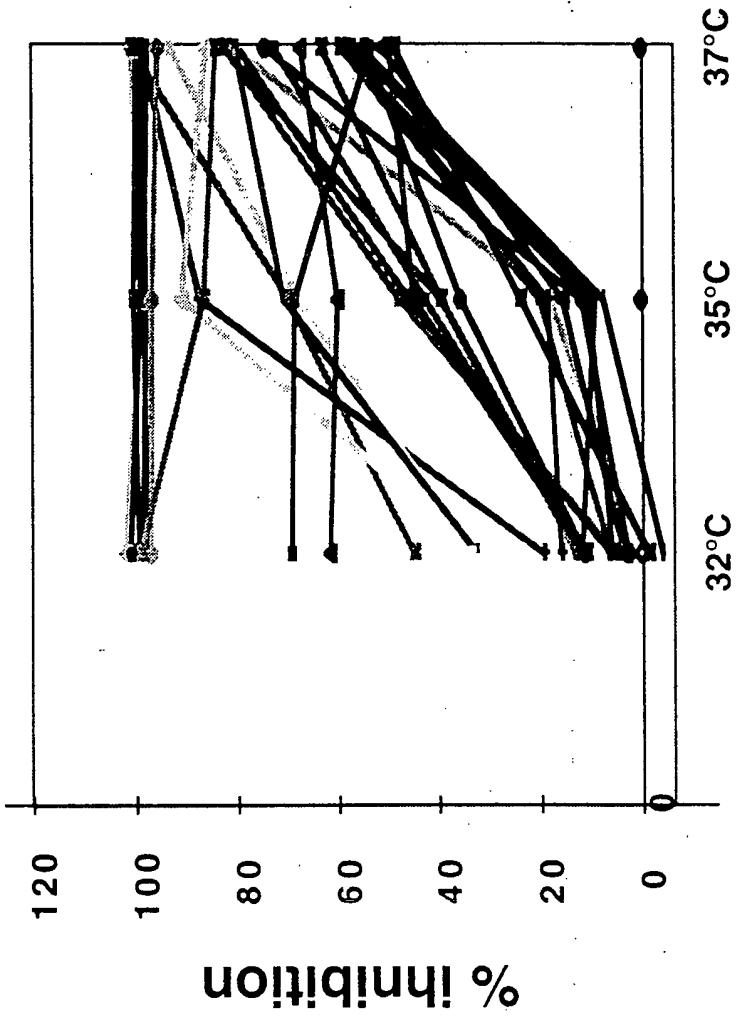
51°C

188

## Temperature-dependent Hypersensitivity

**Strain: NT 99**

Inhibitory effect of  
30 compounds at  
 $32^{\circ}\text{C}$ ,  $35^{\circ}\text{C}$  &  $37^{\circ}\text{C}$

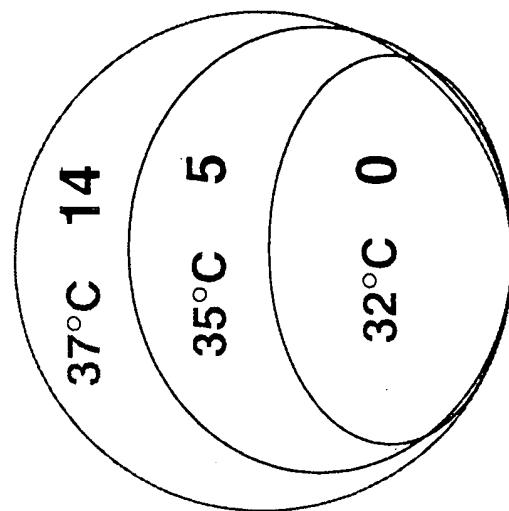


45°C

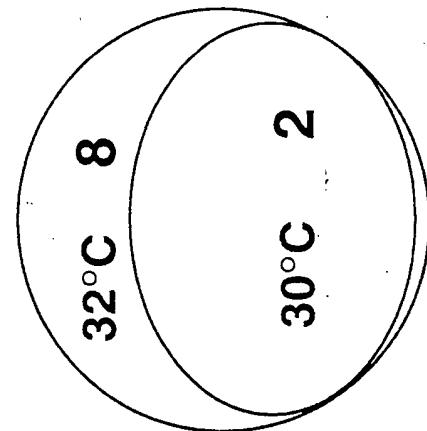
Fig 9



**Hit criteria:** compd that inhibits mutant by  $\geq 50\%$ , and % inh.  
on mutant is higher than on WT by  $\geq 30\%$   
**# of compounds tested:** 480 for NT99; 240 for NT340



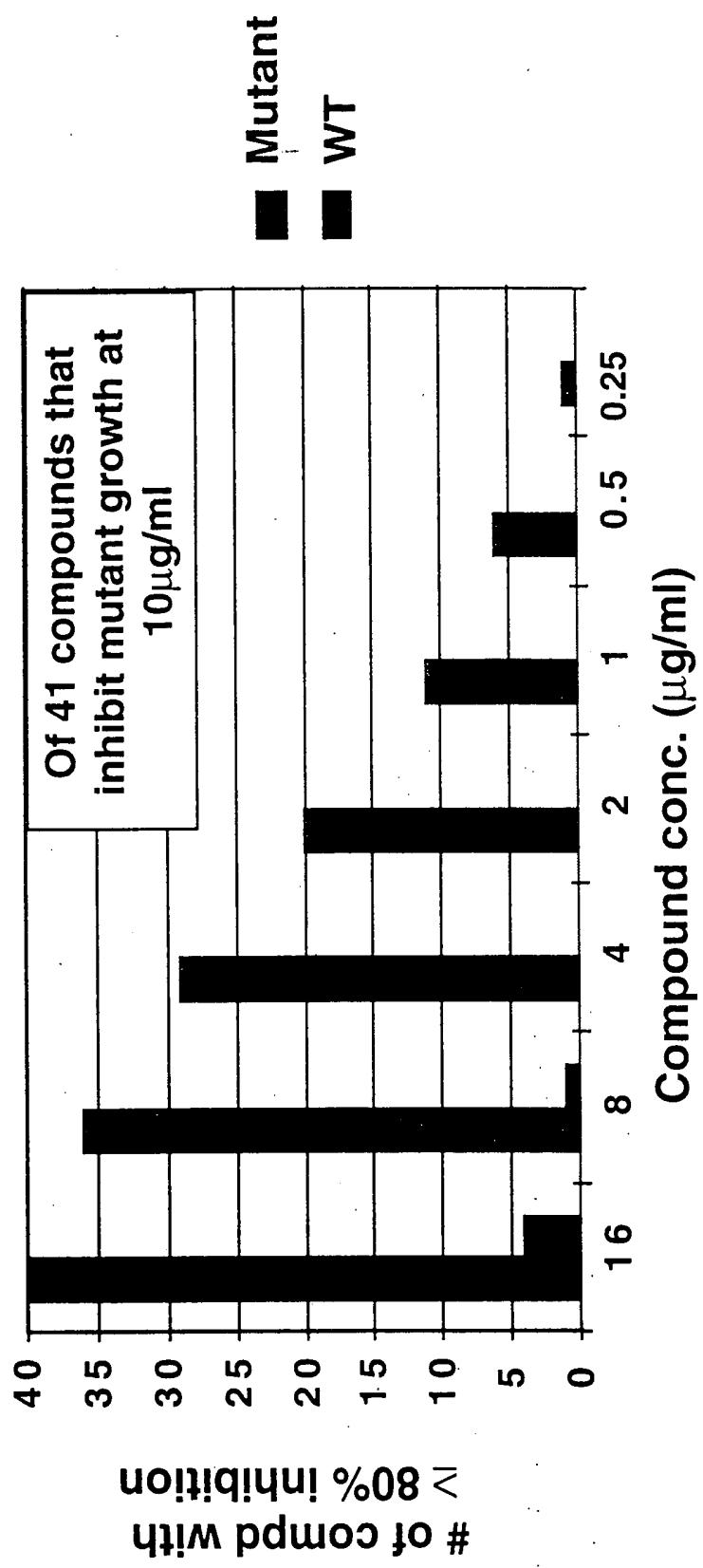
NT99



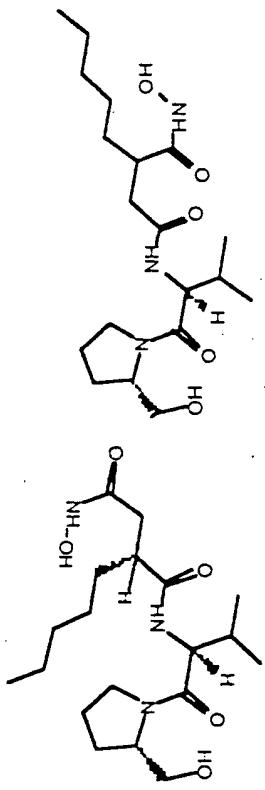
NT340

Fig 10

## Concentration Control of Hit Rate



718 // Structural Similarity of Compounds having  
Identical Mutant Inhibition Profiles.



30-0014

20-0348

F18 12 Pilot Screen With Unknown Inhibitors Against *S. aureus*\*

	20- NT	10- 0157	50- 0817	20- 0116	20- 0204	20- 0860	20- 0123	20- 0287	20- 0045	10- 0373	10- 0752	10- 0197	10- 0341	10- 0797	10- 3775	10- 9370
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\*ND: No data available; blank boxes show no significant difference in MIC from the wild type strain (significance level  $>/-2$ -fold).

Fig 13 Anticipated Mutant Inhibition Profiles

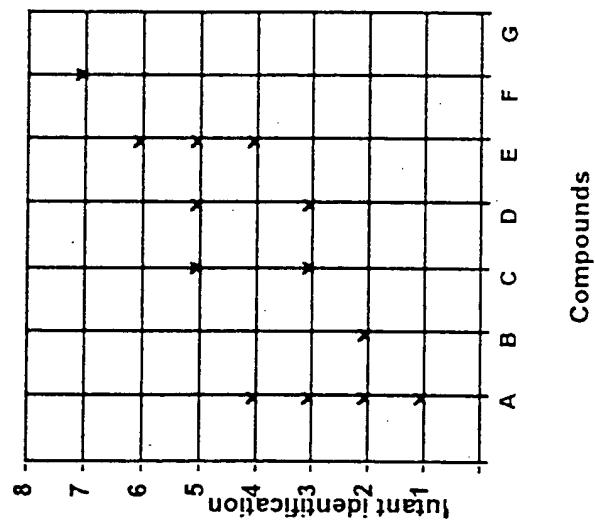
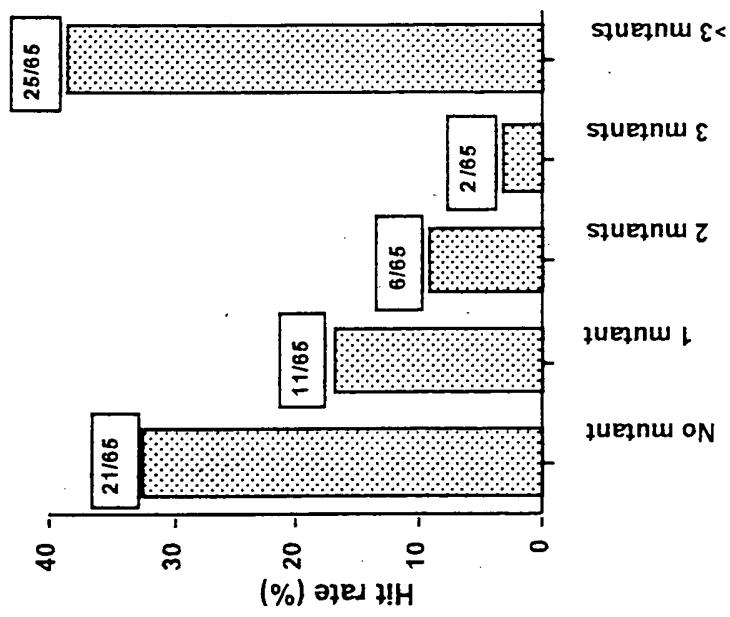


Fig 14 Hit Rate per Multiple of Mutants



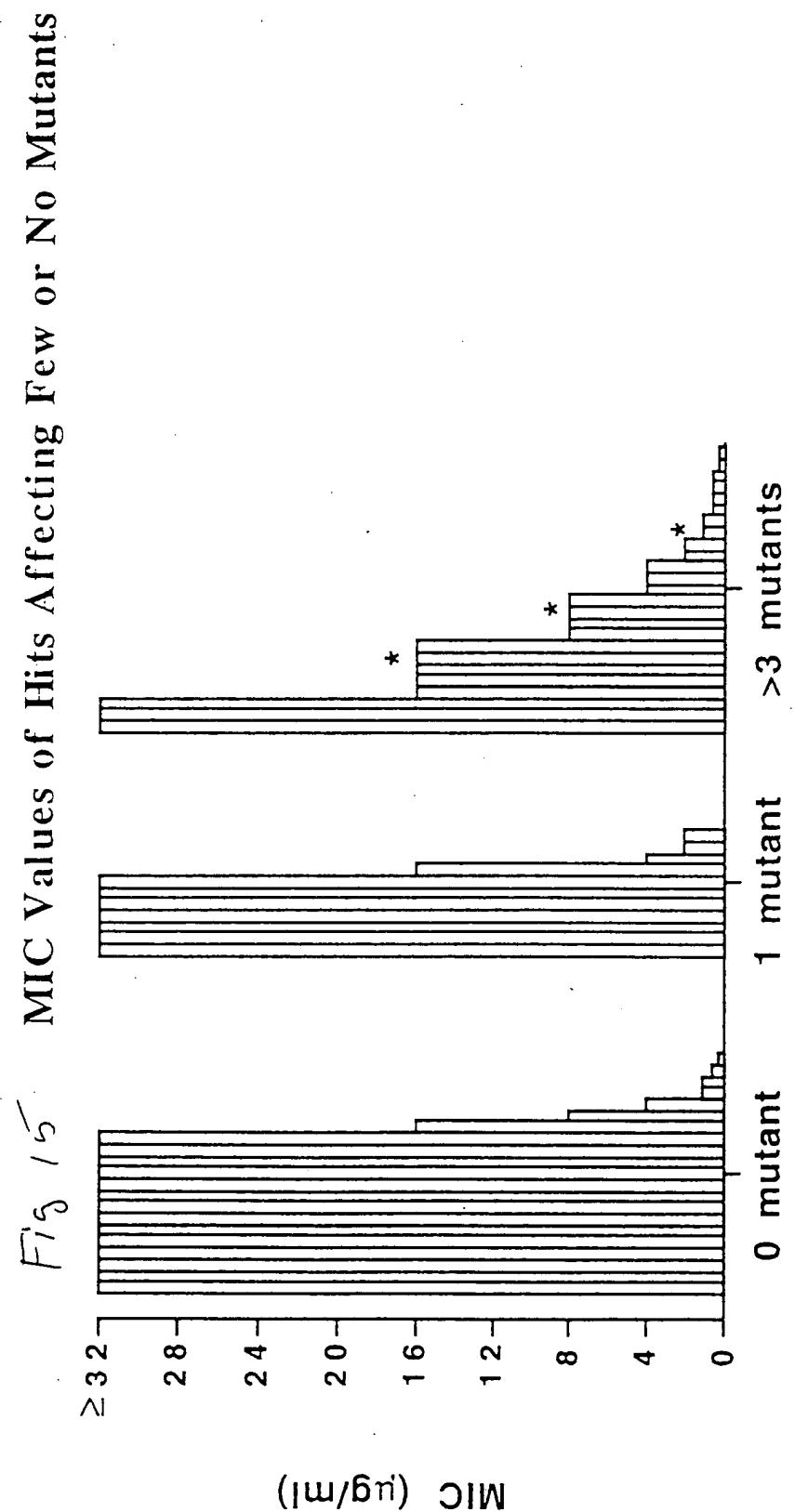
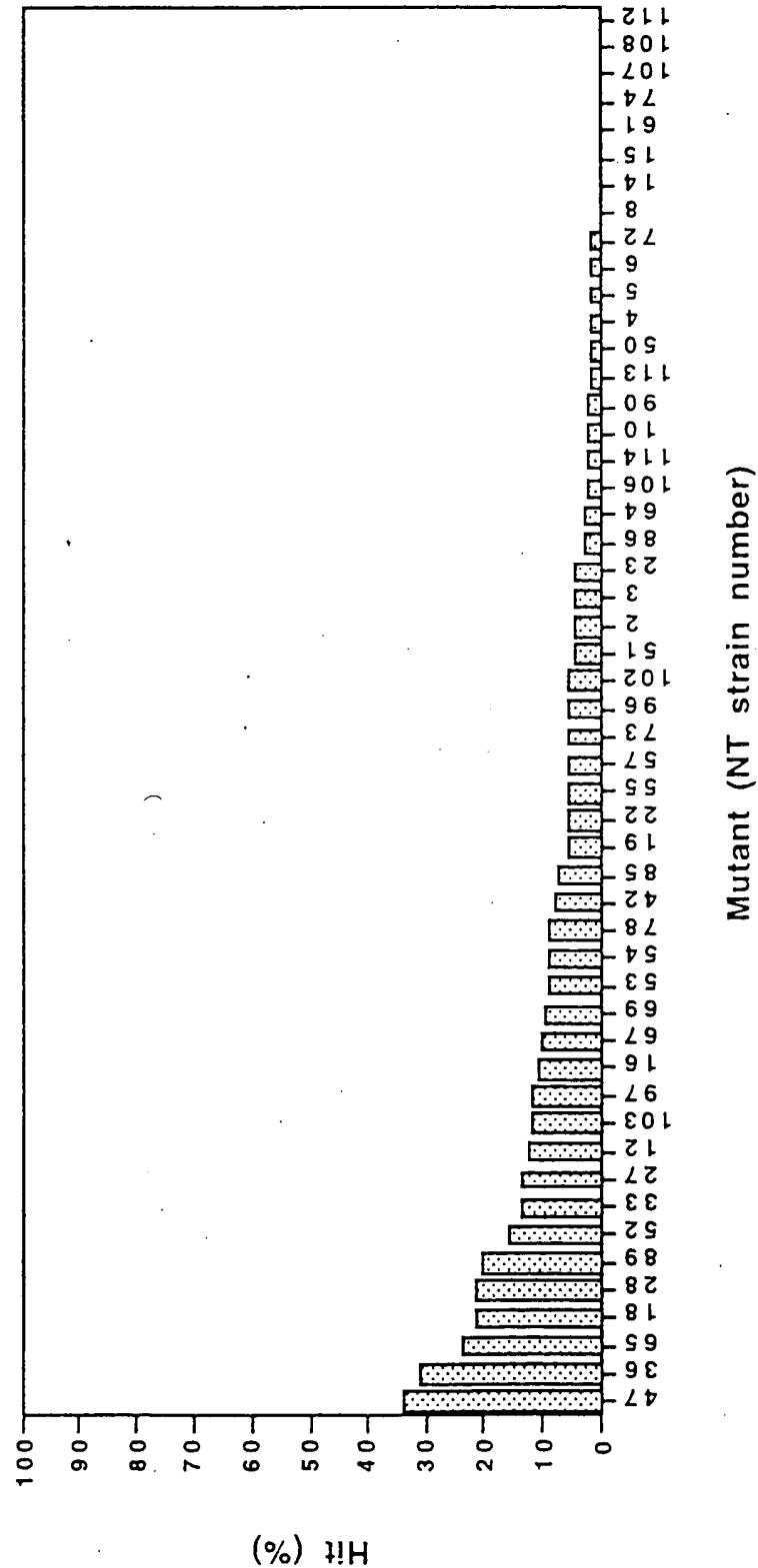


Fig. 16. Relative Number of Hits per Mutant



## Fr 17 Multichannel Screen Advantage

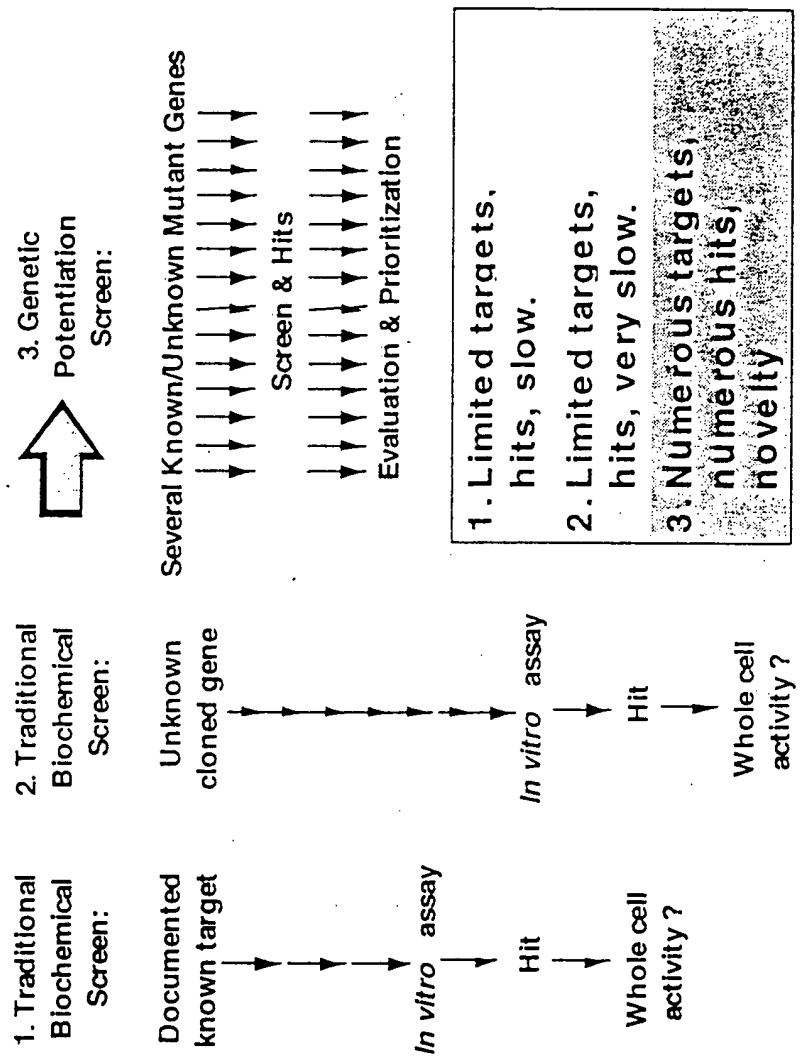


Fig 18 Strategy for Selection of Dominant Lethal Mutants

Relevant Genotype	Phenotype
ts/hypersens	No growth at high temperature
dom	No growth (not viable)
ts/hypersens dom	No growth (not viable)
ts/hypersens dom	Growth at high temperature No growth at low temperature

Fig 19

## Structurally Similar Compounds (continued) 94

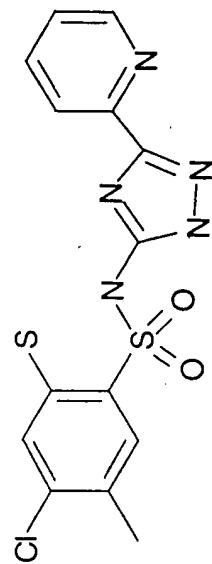
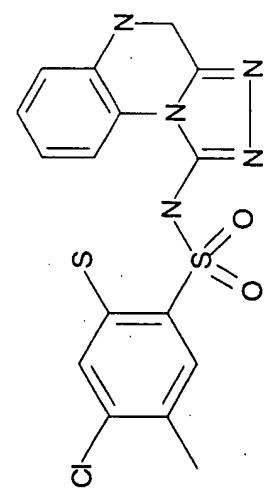
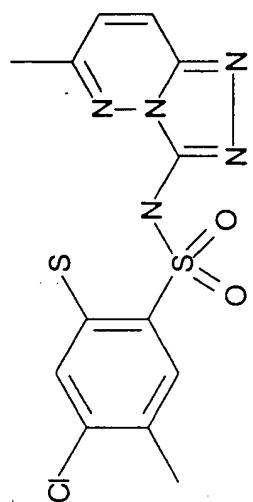
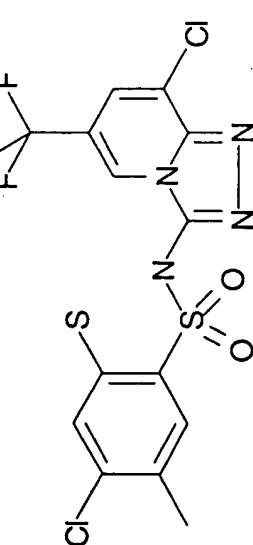
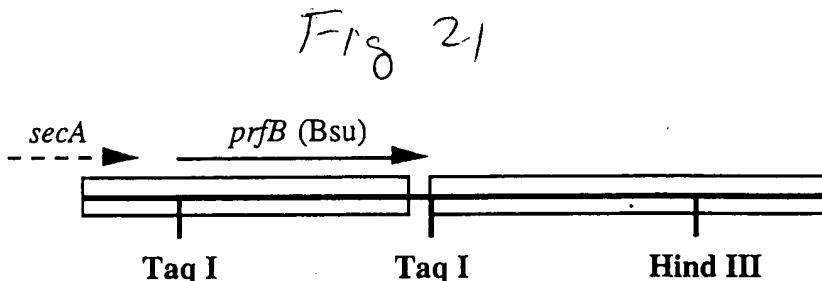


Fig. 20

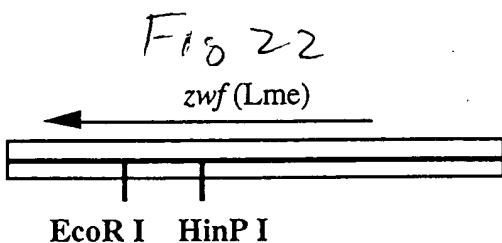


Clone 8135-1011 112ev  
8/21/96

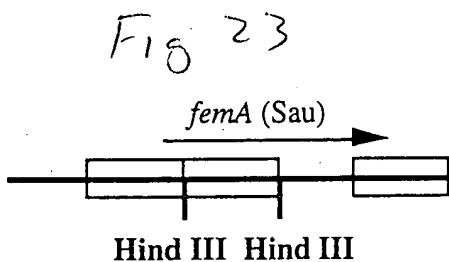
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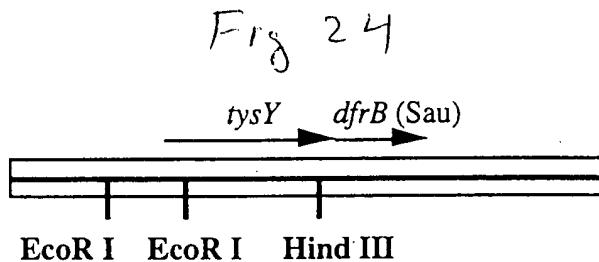
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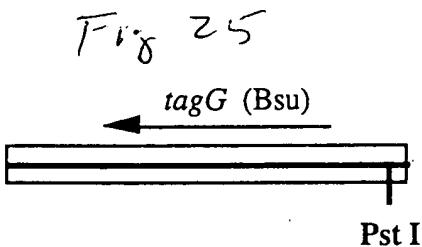
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24 NT8

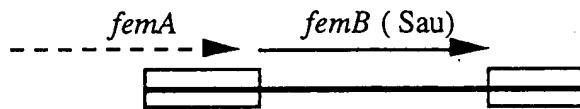


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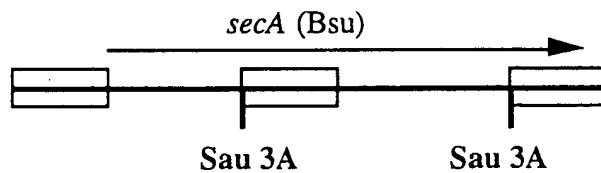
26 NT14

Fig 26



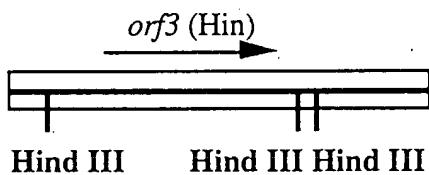
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Fig 27



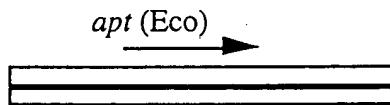
28 NT16

Fig 28



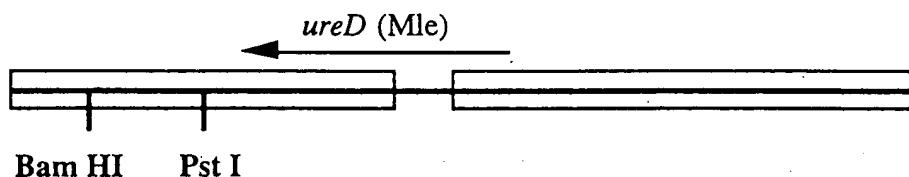
29 NT17

Fig 29

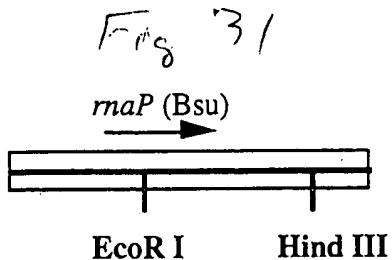


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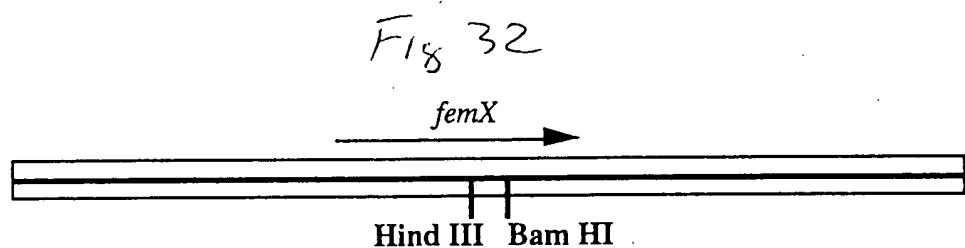
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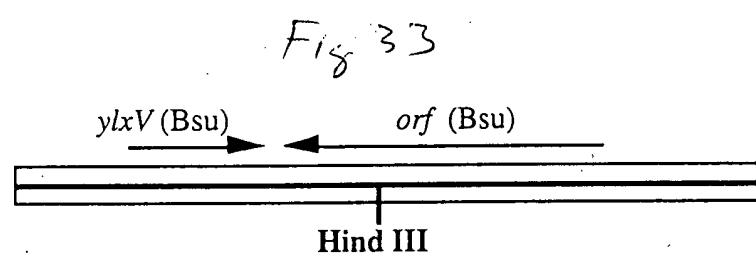
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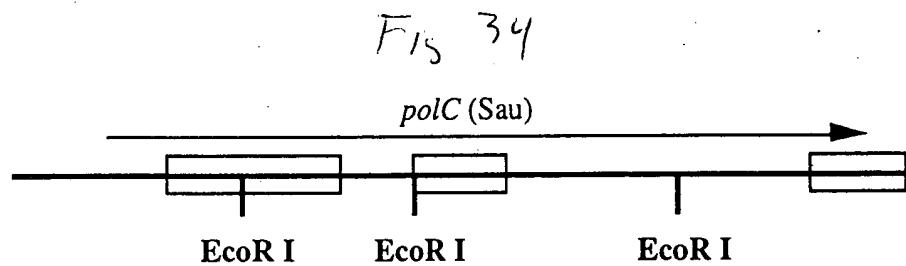
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NT27

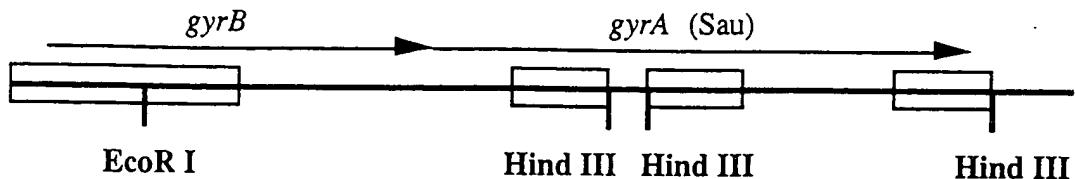


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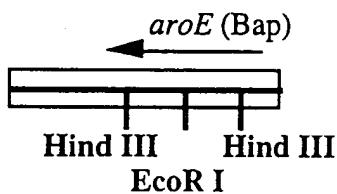
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Fig 35



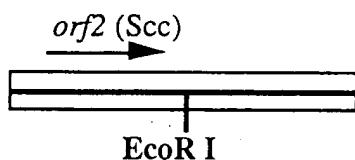
36 NT31

Fig 36



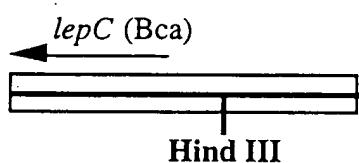
NT33a

Fig 37



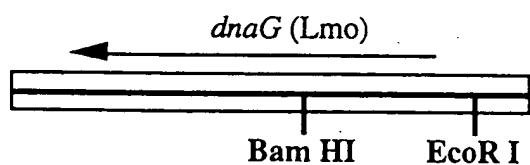
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Fig 38



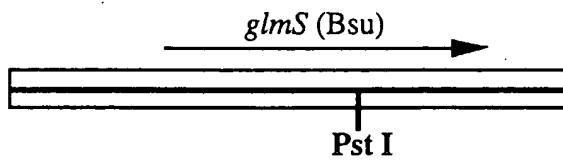
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Fig 39



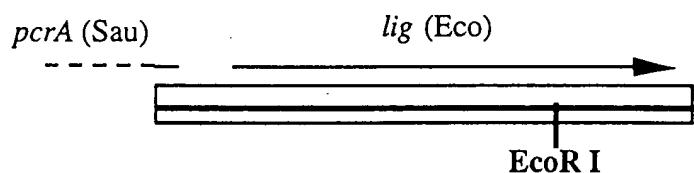
40 NT37

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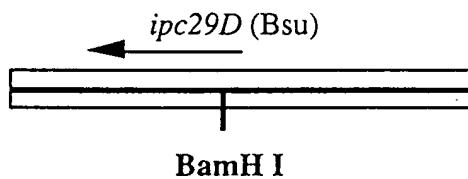
41 NT41/64

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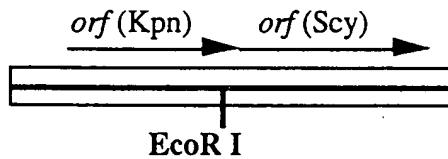
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Fig 42



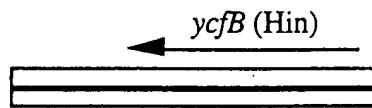
43 NT47

Fig 43



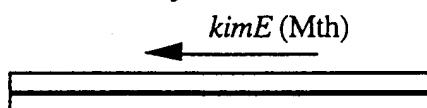
44 NT51

Fig 44



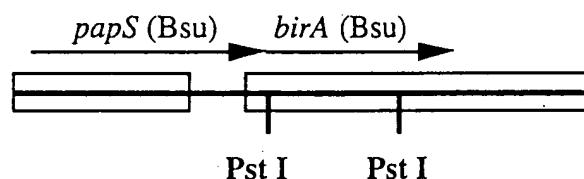
45 NT52

Fig 45



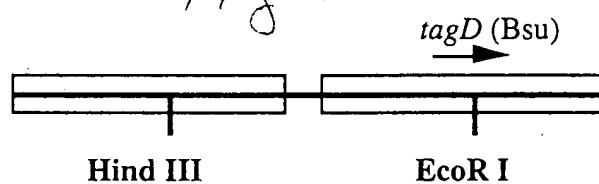
46 NT53

Fig 46



47 NT54

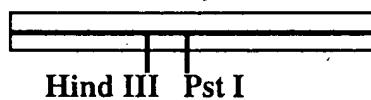
Fig 47



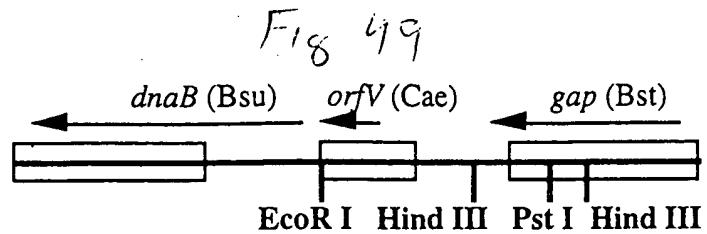
48 NT55

Fig 48

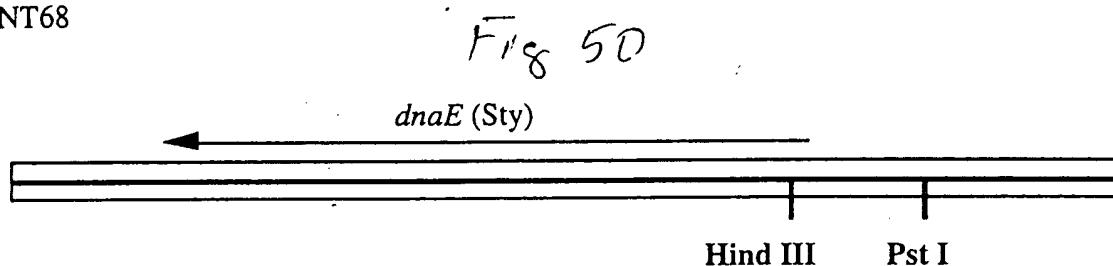
*nadE* (Eco)



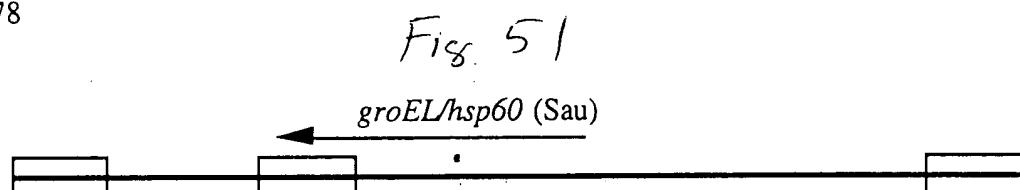
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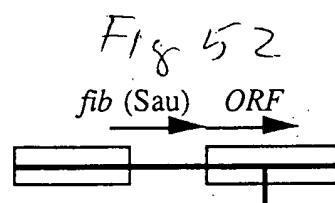
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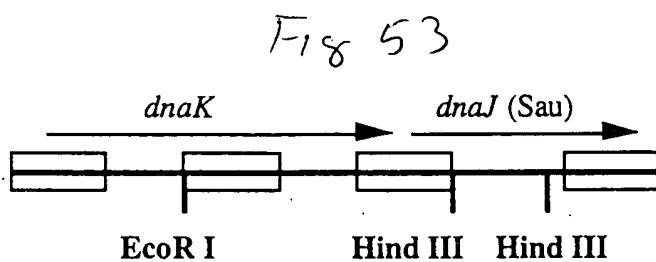
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22 NT81

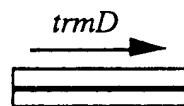


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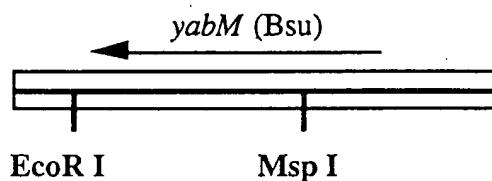
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Fig 54



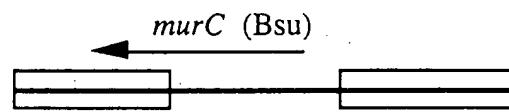
NT94

Fig 55



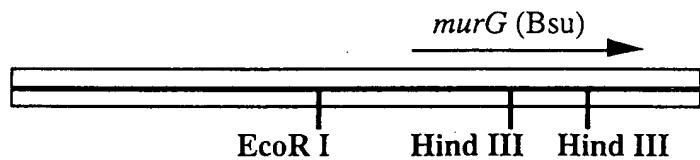
NT96

Fig 56



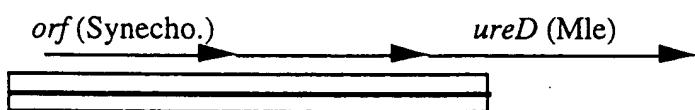
NT99

Fig 57



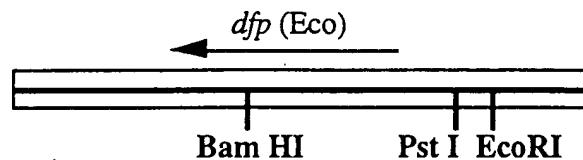
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Fig 58



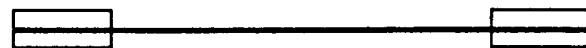
NT114

Fig 59



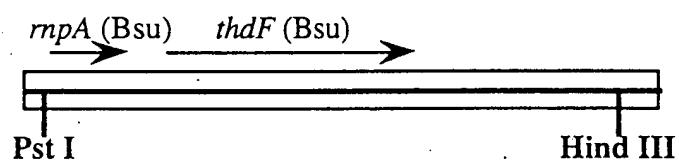
60 NT124

Fig 60



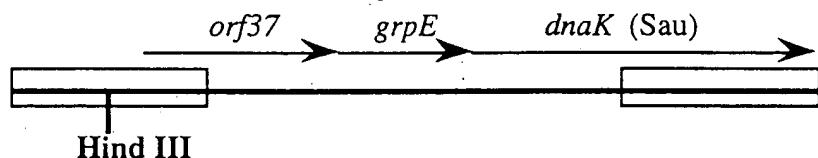
61 NT125

Fig 61



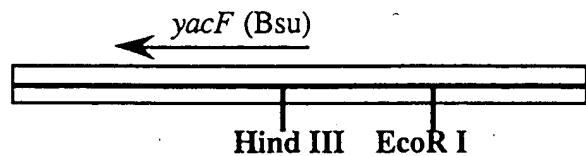
62 NT144

Fig 62



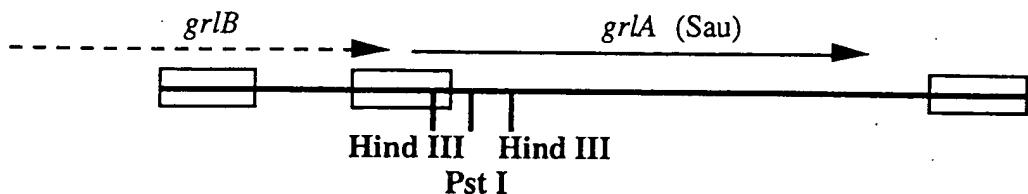
63 NT152

Fig 63



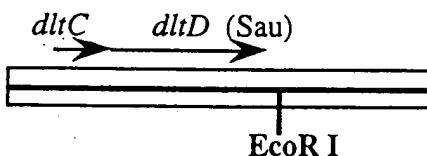
64 NT156

Fig 64



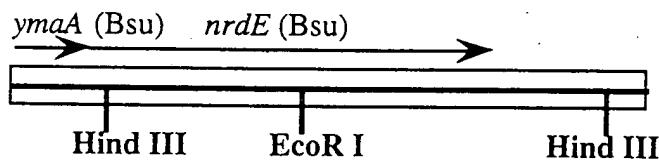
55 NT160

Fig 65



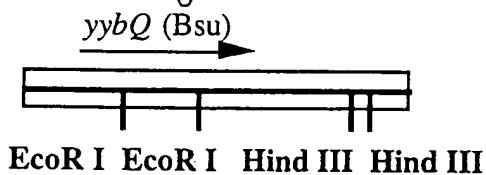
56 NT166

Fig 66

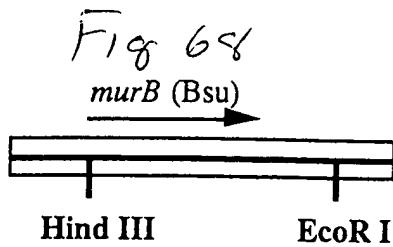


57 NT199

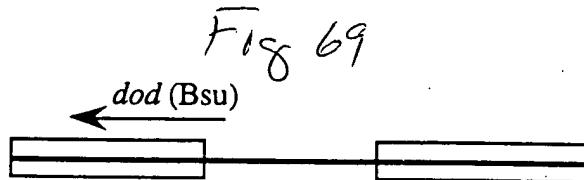
Fig 67



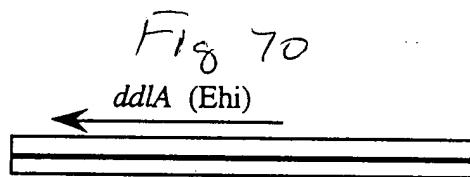
58 NT201



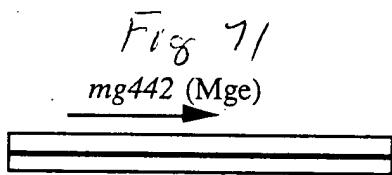
69 NT304



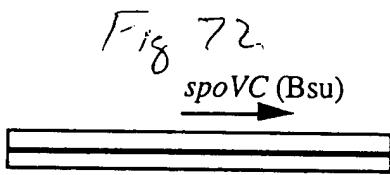
75 NT310



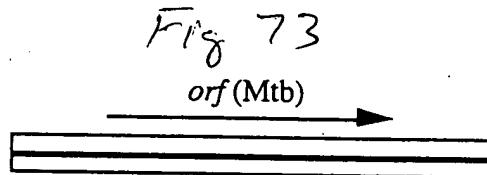
NT312



NT318

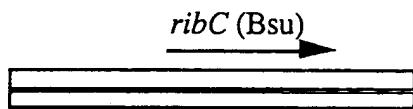


NT321



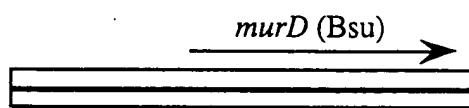
74 NT325

Fig 74



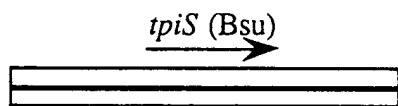
75 NT333

Fig 75



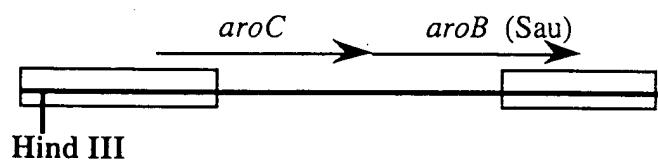
76 NT346

Fig 76



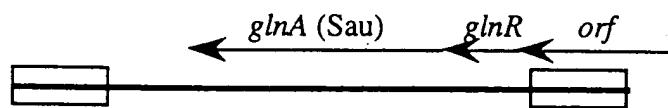
77 NT348

Fig 77

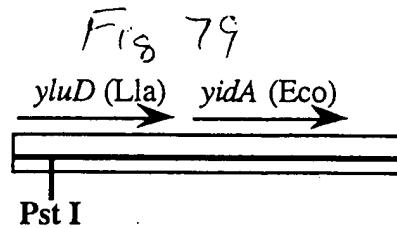


78 NT359

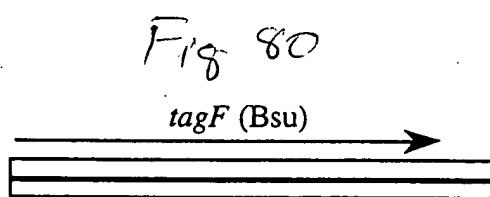
Fig 78



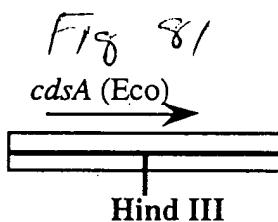
79 NT371



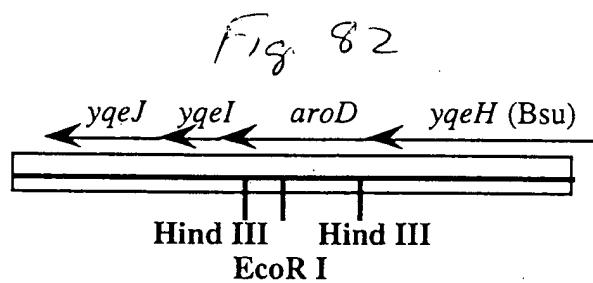
80 NT379



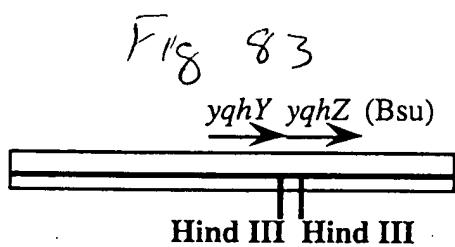
NT380



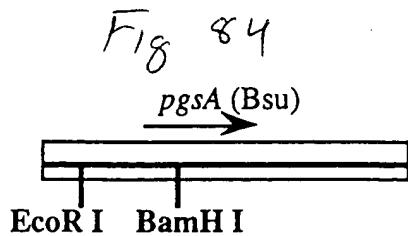
NT401



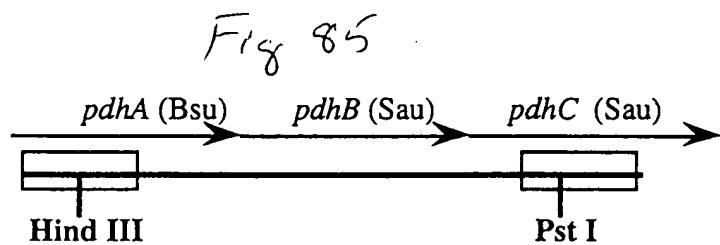
NT423



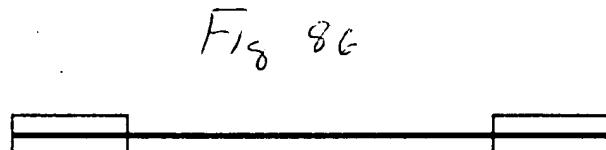
NT432



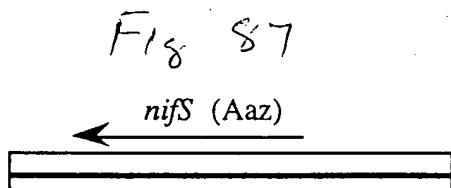
85 NT435



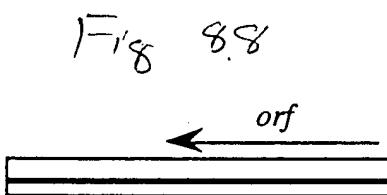
75 NT437



3 - NT438

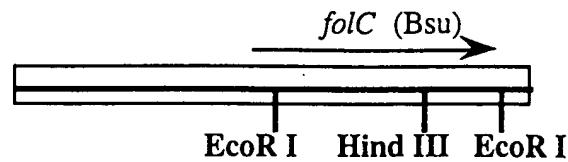


NT462



30 NT482

Fig 89



92 NT486

Fig 90

